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Request for grant of a patent

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1. Your reference	PP/3392 GB	
2. Patent application number *(The Patent Office will fill in this part)*	**9828852.5**	30 DEC 1998
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Nycomed Amersham plc Amersham Place Little Chalfont Buckinghamshire HP7 9NA United Kingdom	
Patents ADP number *(if you know it)*	7377419001	
If the applicant is a corporate body, give the country/state of its incorporation		
4. Title of the invention	NMR Spectroscopy Method	
5. Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	STEVENS HEWLETT & PERKINS 1 Serjeants' Inn Fleet Street LONDON EC4Y 1LL	
Patents ADP number *(if you know it)*	1545003	

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	5
Claim(s)	3
Abstract	
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10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Stevens Hewlett & Perkins
Stevens, Hewlett & Perkins Date 30-12-98

12. Name and daytime telephone number of person to contact in the United Kingdom P Pennant; 0171-936-2499

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NMR SPECTROSCOPY METHOD

This invention is concerned with nuclear magnetic resonance spectroscopy. The technique involves observing the spectrum of a nmr active nuclear species in order to obtain information about the environment in which the species is present. The spectra of nmr active nuclei vary depending on their environment, and this is reported in the literature (PNAS, 93, 12932-6, 1996).

Noble gases having non-zero nuclear spin can be hyperpolarised, i.e. have their polarisation enhanced over the equilibrium polarisation, e.g. by the use of circularly polarised light. Preferred techniques for hyperpolarisation include spin exchange with an optically pumped alkali metal vapour and metastability exchange. Noble gases to which this technique can be applied include helium-3, neon-21, krypton-83, xenon-129 and xenon-131. As described by M S Albert *et al* in US Patent 5,545,396, the technique can be used to prepare hyperpolarised noble gases which can then be administered orally for magnetic resonance imaging of the human body.

It is known that the hyperpolarisation of a noble gas can be transferred by physical contact to another nmr active species. Thus WO 97/37239 describes a method which involves: contacting a sample containing an nmr active nucleus with a hyperpolarised noble gas; scanning the sample using nuclear magnetic resonance spectroscopy, magnetic resonance imaging, or both, in order to detect the nmr active nucleus. It will be understood that magnetic resonance imaging involves determining the presence or location of an nmr active nucleus, and is contrasted with nmr spectroscopy.

The present invention provides a method which comprises:

a) using a hyperpolarised noble gas to effect nuclear

polarisation of an assay reagent comprising at least one nmr active nucleus other than the noble gas,

b) using the assay reagent to perform an assay,

steps a) and b) being performed simultaneously or sequentially in either order, and

c) analysing by nmr spectroscopy an assay reagent comprising the nmr active nucleus.

The hyperpolarised noble gas is helium-3, neon-21, krypton-83, xenon-129 or xenon-131, preferably helium-3 or xenon-129, as these are now commercially available at high isotope purity with a high degree of hyperpolarisation and an adequate half life.

Nmr active nuclei are those having non-zero nuclear spin and include hydrogen, carbon-13, nitrogen-15, fluorine-19, silicon-29 and phosphorus-31. Of these carbon-13 is preferred. Carbon-13 is present at a natural abundance (relative to carbon-12) of about 1%. Just as the labelling of organic compounds with carbon-14 is widely practised, so organic compounds can be labelled or enriched with carbon-13, either generally or at specific positions in the molecule. Preferably organic compounds for use in this invention contain carbon-13, either generally or at least one specific position, at an abundance of at least 5%, preferably at least 90% and ideally at approaching a 100%. By the method of this invention, carbon-13-labelled compounds can provide all the information provided by corresponding carbon-14-labelled compounds and much more besides.

An assay reagent is a substance or compound that takes part in an assay, by being introduced as an initial reagent or by being formed *in situ* and perhaps transiently during the assay, or by being formed as a product of the assay. An assay is a test performed partly or wholly *ex vivo* in which a physical or chemical change involving a biological species is observed. A biological species is one which is present in living systems or which is introduced into and is reactive with such systems.

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In step a), a hyperpolarised noble gas is used to effect nuclear polarisation of an assay reagent comprising at least one nmr active nucleus other than the noble gas. This polarisation may be effected by physical contact. The hyperpolarised gas may be in the gas phase, or may alternatively be liquid e.g. by being dissolved in a lipid or fluorocarbon solvent, or a solid e.g. by being adsorbed on to a solid surface. The assay reagent may be solid but is generally fluid. A hyperpolarised gas may be bubbled into a fluid assay reagent. Or a hyperpolarised gas solution may be mixed with a fluid assay reagent. The hyperpolarised gas may be cooled and/or maintained in a magnetic field to preserve the hyperpolarisation. Similarly the resulting assay reagent comprising at least one polarised nmr active nucleus may preferably be cooled and/or maintained in a magnetic field in order to preserve the polarisation.

Preferably, step c) is performed by examining the assay reagent using both nmr spectroscopy to obtain more than one spectrum, and magnetic resonance imaging to obtain one or more discrete spectral location, and repeating the examination at least once so as to obtain quantitative information about kinetic or time-dependant alteration in chemistry, environment or structure of the assay reagent

Assays envisaged according to this invention include competition assays, binding assays, immunoassays, hybridisation assays, test involving macro-organisms, and binding studies performed on tissue sections, cultured cells or micro-organisms. Preferred examples are discussed in the following paragraphs.

Hybridisation assays are very widely used for sequencing and for detection of point or deletion mutations. When a conventionally labelled polynucleotide probe is hybridised with a polynucleotide target, analysis of the melting temperature or other property of the hybrid can give some limited information about the nucleotide sequence of the target. According to the present invention, a probe labelled with a polarised nmr active nucleus can give different and more extensive information about the target

than has previously been available. This is because a polarised nmr active nucleus generates an nmr spectrum which is dependent on its environment; and that environment extends beyond the labelled molecule itself to other molecules in the immediate vicinity. Thus for example a nucleotide labelled with polarised carbon-13, when incorporated in a single stranded polynucleotide, can give information about two or more adjacent nucleotide residues in the chain. When that labelled polynucleotide probe is hybridised with a polynucleotide target, nmr spectroscopic analysis of the nmr carbon-13 label can give information, not only about the complementary nucleotide residue in the target, but also about two or more adjacent nucleotide residues in the target.

Nucleosides or nucleotides or nucleotide analogues can readily be enriched with carbon-13 at one or several specified points in the molecule. Polarisation of the carbon-13, by contact with a hyperpolarised noble gas, may be effected either before, during or after incorporation of the monomer into a polynucleotide; and before, during or after hybridisation of that polynucleotide with a complementary strand.

Many assays involve a reaction in which a chemical bond is broken. According to an aspect of the present invention, an assay reagent is an organic compound comprising one or more nmr active nuclei associated with a bond which is broken during the course of the assay. In the case of a single nmr active nucleus this is located preferably at the actual site of the breaking of the chemical bond such that the change in local environment of the active nucleus subsequent to the bond breaking will give rise to a change in the spectrum of the nmr active nucleus. The nmr spectra of two or more active nuclei will be different, depending on whether they are present within the same molecule or in different molecules. When two or more active nmr nuclei are in an appropriate proximity to one another they are said to be spin coupled. This gives rise to a distinct nmr spectrum which can be monitored. It is therefore possible to analyse by nmr spectroscopy the rate and extent of the bond's breaking

by the disruption of the spin coupling. In this and other assays, the assay reagent may be analysed repeatedly by nmr spectroscopy so as to generate information about a change with time of the assay reagent.

In another aspect of the invention, an assay reagent may be administered to a macro-organism, e.g. a human or animal, and nmr spectroscopic analysis performed of excreta, e.g. urine, faeces or breath, of the macro-organism. In this case, the assay reagent may be labelled by contact with a hyperpolarised gas before administration; or alternatively the excreta may be labelled before spectroscopic analysis.

In yet another aspect of the invention, an assay reagent may be used in binding studies on bacteria or other eukaryotic or prokaryotic micro-organisms or cultured cells.

Assays according to this invention may conveniently be carried out in multiwell plates. An assay reagent in each well may be labelled by contact with a hyperpolarised noble gas, prior to addition of other assay reagents. Or an assay reagent in bulk may be labelled by contact with a hyperpolarised noble gas prior to being dispensed into individual wells of a multiwell plate. In many cases, assays can be performed in a homogenous mode, that is to say without the need for a separation step to remove one fraction of the labelled reagent.

In addition in cases where the spectra of the ^{13}C labelled assay components are distinct from one another more than one assay may be performed and simultaneously monitored in a single well or spot of a multi-assay array. This would allow multiplexing of several related or unrelated assays in parallel within a single well or spot in a multi-assay array which is either ordered or random. In addition the technique may be applied to aerosol droplets where no well, container or surface is used to contain the assay and to analysis of samples in flow-through devices.

CLAIMS

1. A method which comprises:

a) using a hyperpolarised noble gas to effect nuclear polarisation of an assay reagent comprising at least one nmr active nucleus other than the noble gas,

b) using the assay reagent to perform an assay,

steps a) and b) being performed simultaneously or sequentially in either order, and

c) analysing by nmr spectroscopy an assay reagent comprising the nmr active nucleus.

2. The method of claim 1, wherein step c) is performed by examining the assay reagent using both nmr spectroscopy to obtain more than one spectrum, and magnetic resonance imaging to obtain one or more discrete spectral location, and repeating the examination at least once so as to obtain quantitative information about kinetic or time-dependant alteration in chemistry, environment or structure of the assay reagent

3. The method of claim 1 or claim 2, wherein the nmr active nucleus is ^{15}N, ^{19}F, ^{31}P or ^{13}C.

4. The method of claim 3, wherein the assay reagent is a compound of which at least one atom contains an artificially high concentration of the nmr active nucleus.

5. The method of any one of claims 1 to 4, wherein the assay reagent is a nucleotide, or nucleotide analogue, polynucleotide, amino acid, amino acid analogue, polypeptide or protein.

6. The method of any one of claims 1 to 5, wherein the assay is a nucleic acid hybridisation assay.

7. The method of any one of claims 1 to 5, wherein the assay is a binding assay.

8. The method of any one of claims 1 to 7, wherein the assay reagent is an organic compound comprising one or more nmr active nuclei associated with a bond which is broken during the course of the assay.

9. The method of claim 8, wherein the assay reagent contains two or more nmr active nuclei present in a spin coupled state and giving rise to distinct spectra, an assay performed resulting in a change in chemical structural or environmental status of the assay reagent, and the changes are monitored.

10. The method of any one of claims 1 to 9, wherein the assay reagent is analysed repeatedly in step c) so as to generate information about a change with time of the assay reagent.

11. The method of any one of claims 1 to 10, wherein an organic compound specifically labelled with an nmr active nucleus is administered to a micro-organism, macro-organism or cultured cells and an excretion product of the labelled compound is analysed by nuclear magnetic resonance spectroscopy, nuclear magnetic resonance imaging or both.

12. The method of any one of claims 1 to 10, wherein the assay is a binding study performed using micro-organisms or cultured cells.

13. The method of any one of claims 1 to 10, wherein the assay

is performed in a homogenous mode.

14. The method of any one of claims 1 to 13, wherein more than one assay is multiplexed and monitored by nmr spectroscopy and optionally magnetic resonance imaging.

15. The method of any one of claim 1 to 14, wherein the assay is performed in a multiwell or multispot assay array.

16. The method of any one of claim 1 to 14, wherein step c) is performed in an aerosol or flow-through device applied to aerosol droplets where no well, surface or container is used to contain the assay reagent.

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